Antinociceptive and Antihyperalgesic Effects of Tapentadol in Animal Models of Inflammatory Pain

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ABSTRACT

The novel analgesic tapentadol HCl [(--)-(1R,2R)-3-(3-dimethylamino)-1-ethyl-2-methyl-propyl)-phenol hydrochloride] combines µ-opioid receptor (MOR) agonism and noradrenaline reuptake inhibition (NRI) in a single molecule and shows a broad efficacy profile in various preclinical pain models. This study analyzed the analgesic activity of tapentadol in experimental inflammatory pain. Analgesia was evaluated in the formalin test (pain behavior, rat and mouse), carrageenan-induced mechanical hyperalgesia (paw-pressure test, rat), complete Freund’s adjuvant (CFA)-induced paw inflammation (tactile hyperalgesia, rat), and CFA knee-joint arthritis (weight bearing, rat). Tapentadol showed antinociceptive activity in the rat and mouse formalin test with an efficacy of 88 and 86% and ED50 values of 9.7 and 11.3 mg/kg i.p., respectively. Tapentadol reduced mechanical hyperalgesia in carrageenan-induced acute inflammatory pain by 84% with an ED50 of 1.9 mg/kg i.v. In CFA-induced tactile hyperalgesia, tapentadol showed 71% efficacy with an ED50 of 9.8 mg/kg i.p. The decrease in weight bearing after CFA injection in one knee joint was reversed by tapentadol by 51% with an ED50 of 0.9 mg/kg i.v. Antagonism studies were performed with the MOR antagonist naloxone and the α2-noradrenergic receptor antagonist yohimbine in the carrageenan- and CFA-induced hyperalgesia model. In the CFA model, the serotonergic receptor antagonist ritanserin was also tested. The effect of tapentadol was partially blocked by naloxone and yohimbine and completely blocked by the combination of both, but it was not affected by ritanserin. In summary, tapentadol showed antinociceptive/antihyperalgesic analgesic activity in each model of acute and chronic inflammatory pain, and the antagonism experiments suggest that both MOR activation and NRI contribute to its analgesic effects.

Introduction

Pain is often associated with inflammation, which results from tissue damage, chemical stimuli, or autoimmune processes. These stimuli induce the release of inflammatory mediators (prostaglandins, bradykinin, histamine, growth factors, and neurogenic factors) (Kidd and Urban, 2001; Kidd et al., 2004), which leads to sustained activation and sensitization of primary nociceptors and higher-order neurons involved in the transmission of the nociceptive input (Marchand et al., 2005).

These processes can lead to central sensitization and hypersensitivity. For example, in rats with knee joint inflammation, spinal nociceptive neurons show an increased response to innocuous and noxious mechanical stimulation of the inflamed joint (Schaible et al., 1987, 2009). Central sensitization can also lead to persistent hypersensitivity of dorsal horn neurons in monoarthritic and polyarthritic rats (Menetrey and Besson, 1982). These functional alterations on the cellular level probably contribute to the mechanical and tactile hyperalgesia observed in carrageenan- and complete Freund’s adjuvant (CFA)-induced inflammatory pain models.

Thus, inflammatory pain is a complex pain condition, and it is often insufficiently treated because the available treatment options have limited efficacy and/or poor tolerability, in particular in chronic conditions such as rheumatoid arthritis and osteoarthritis (Zhang et al., 2010).

Agonists at the µ-opioid receptor (MOR) are an important option for the treatment of moderate to severe pain. However, although MOR agonists are very effective in acute pain, their efficacy may be limited in chronic pain conditions, including chronic inflammatory pain, mainly because of limited tolerability (Portenoy, 1996; Mao et al., 2000; Martin and Eisenach, 2001; Kalso et al., 2004; Fitzcharles et al., 2010; Lang et al., 2010).

Alternative treatment options in chronic pain, in particular inflammatory pain, are nonsteroidal anti-inflammatory drugs or selective cyclooxygenase-2 (COX-2) inhibitors. How-
ever, both drug classes may not have adequate analgesic efficacy, particularly in arthritic disorders (Zhang et al., 2010). Furthermore, they are associated with gastrointestinal adverse effects, renal problems, increased risk of impaired blood coagulation with subsequent bleeding (Langford, 2006), and the risk of cardiac side effects, in particular with high-dose, long-term treatment (Chen et al., 2008). Thus, there is a need for drugs that are effective in chronic pain, including chronic inflammatory pain, with an improved tolerability profile.

There is good evidence that inflammation-induced central sensitization can be counteracted through descending inhibitory pathways, the activity of which is increased during inflammation (Schaible et al., 1991), suggesting that in inflammatory pain the noradrenergic descending inhibitory system plays an important role in pain modulation.

Tapentadol HCl [(−)-(1R,2R)-3-(3-dimethylamino)-1-ethyl-2-methyl-propyl]-phenol hydrochloride] is a novel, centrally acting analgesic that combines MOR agonism with NRI in a single molecule and induces potent and efficacious analgesia in the clinic with an improved tolerability profile compared with classic MOR agonists, such as morphine or oxycodone (Hale et al., 2009; Lange et al., 2010). Preclinically, tapentadol shows potent analgesia in rodent models of acute nociceptive and chronic neuropathic pain (Tzschtentke et al., 2006, 2007, 2009; Schröder et al., 2010). To further explore the efficacy profile of tapentadol, the aim of this study was to assess the effects of tapentadol in animal models of acute and chronic inflammatory pain. In previous studies we established, in nociceptive and neuropathic pain models, that both MOR agonism and NRI contribute to the analgesic effects of tapentadol (Tzschtentke et al., 2007; Schröder et al., 2010). Recently, we also showed that these two mechanisms of tapentadol show an intrinsic synergistic interaction (Schröder et al., 2011). Therefore, in additional studies, the contribution of MOR agonism and NRI to the effects of tapentadol in inflammatory pain was evaluated in two exemplary inflammatory pain models by means of antagonism studies with the MOR antagonist naloxone and the α₂-agonist yohimbine.

Materials and Methods

Behavioral Studies

Animals. The studies were conducted with male NMRI mice (20–35 g) and male Sprague-Dawley rats (130–180 g), supplied by commercial breeders (Charles River, Sulzfeld, Germany; Iffa Credo, Brussels, Belgium; Janvier, Genest St. Isle, France). Animals were housed under a 12-h light/dark cycle (lights on at 6:00 AM) with room temperature 20–24°C, relative air humidity 35 to 70%, 15 air changes per hour, and air movement ~0.2 m/s. The animals had free access to standard laboratory food (Ssniff R/M-Haltung; Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. All animals were used only once in all pain models. There were at least 5 days between delivery of the animals and the start of the experiment.

Animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983) and the German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is also an ethics committee.

Experimental Procedures. Animals were assigned randomly to treatment groups. Different doses and vehicle were tested in a randomized fashion. Although the operators performing the behavioral tests were not formally blinded with respect to the treatment, they were not aware of the study hypothesis or the nature of differences between drugs.

Formalin test in mice. The formalin test is a broadly used model of chemically induced persistent pain (Dubuisson and Dennis, 1977). Formalin injection into a rat or mouse hindpaw induces a spontaneous nociceptive behavior such as flinching, licking, or biting of the paw. Generally two phases of the nociceptive response can be observed in this model: a first phase starting immediately after injection and a second phase starting approximately 15 min after formalin injection. In the first phase nociceptors are activated directly by the chemical stimulation of formalin; therefore, this period is considered as a model of acute pain. The second phase reflects a spinal and peripheral hypersensitization (chronic phase) and is considered as a model for chronic pain. In this study, a period within the second phase was analyzed.

The test was carried out in a Plexiglas box with a mirror placed behind it to allow an unobstructed view of the animals. Each animal was injected with 20 μl of 1% formalin in 0.9% NaCl subcutaneously into the dorsal surface of the right hindpaw. After placing the mice back into the chamber the nociceptive behavior was measured by observation for 21 to 24 min postformalin, and the amount of time spent licking and biting the injected paw was counted (Dubuisson and Dennis, 1977). A vehicle control group was included for each investigation. The group size was 10 animals.

Formalin test in rats. Nociceptive behavior was induced in rats by subcutaneous injection of 50 μl of 5% formalin in 0.9% NaCl into the dorsal surface of the right hindpaw. The observation period started 21 min after the injection (21st to 27th min). Characteristic pain behavior was scored (0, normal behavior; 1, paw lifting; 2, flinching; 3, licking and biting of the injected paw) and multiplied by its length of time (s) during the observation period. The group size was 10 animals.

Carrageenan-induced inflammatory pain in rats. This model of acute inflammatory pain was conducted according to Randall and Selitto (1957). Acute inflammation was induced by injection of 0.1 ml of carrageenan solution (0.5% in distilled water) subcutaneously into the plantar surface of the right hind paw of the rat. The mechanical nociceptive threshold was measured using an Algesiometer (Ugo Basile, Comerio, Italy). The device generates a mechanical force with a linear increase over time. The force was applied to the dorsal surface of the inflamed hind paw via a cone-shaped stylus with a rounded tip (3 mm²). The nociceptive threshold (T̄) was defined as the force at which the rat vocalized (cutoff force 450 g). Compounds or vehicle were given 3 h after carrageenan injection. The mechanical nociceptive threshold was measured 15, 30, 45, 60, 90, and 120 min after drug or vehicle administration. The drug effects are expressed as percentages of the maximal possible effect based on the following formula: percentage of the maximal possible effect = (T̄ control − T̄ drug)/(cutoff − T̄ control) × 100. The group size was 12 animals.

Complete Freund’s adjuvant-induced hyperalgesia in rats. CFA-induced hyperalgesia is frequently used as an animal model to study chronic inflammatory pain (Pearson and Wood, 1963; Colpaert, 1987). The CFA-induced inflammation is accompanied by a tactile hyperalgesia, which is robust over several days (Ma and Woolf, 1996). Rats received a single intraplantar injection of 100 μl of a 1 mg/ml dose of heat-killed and dried Mycobacterium tuberculosis (H37 Ra) in a mixture of paraffin oil and an emulsifying agent, mannide monoleate (CFA). Animals injected with saline served as controls. One day after CFA injection, mechanical hyperalgesia was tested with electronic von Frey hairs (Somedic Sales AB, Hörby, Sweden). Animals were placed in a plastic cage with a wire-mesh bottom that allowed full access to the paws. Behavioral accommodation was allowed for 30 min. The tactile hyperalgesia was tested as tactile withdrawal threshold before and 15, 30, and 60 min after drug administration. Results are presented as percentage of change to prevalue, comparing thresholds of the CFA-injected paw and control.
threshold of un.injected contralateral paw before drug administration. The group size was 10 animals.

Rheumatoid arthritis model of chronic inflammatory pain in rats. For the knee joint arthritic model of chronic inflammatory pain used in this study, CFA was injected into one knee, resulting in a localized inflammatory response (monoarthritis) that was restricted to the knee joint of one hind limb (Butler et al., 1992). The advantage of this method is that it avoids systemic changes and illness, which could affect behavior, and generally develops after CFA injection into the tail or hindpaw in chronic studies for polyarthritis. The intra-articular CFA injection results in the pathophysiology of joint pain, which constitutes a chronic model for inflammatory joint pain, closely resembling the pathology in humans (Wilson et al., 2006).

Rats were anesthetized using 3% isoflurane in oxygen. The left knee was cleaned using a CutaseptP solution and then injected with 150 μl of CFA, containing 2 mg/ml inactivated and dried M. tuberculosis. The right joints remained untreated. Animals were assessed for changes in weight bearing 5 days after intra-articular injection.

Naive rats distribute their body weight equally between their two hind legs. Induction of knee joint arthritis of the left knee results in a decreased weight bearing on the affected hind leg. Weight bearing on each hind leg was determined using a rat incapacitance tester (Somedic Sales AB). Rats were placed in an angled Plexiglas chamber of the incapacitance tester with their hind paws on separate sensors, and the percentage of body weight distribution was calculated over 30 s. Data were expressed as percentage of contralateral weight bearing, with 100% values resulting from equal weight distribution across both hind limbs.

Antagonism Studies

The MOR antagonist naloxone (1 mg/kg), the α2-adrenoceptor antagonist yohimbine (2.15 mg/kg), and the 5-HT2 receptor antagonist ritanserin (0.316 mg/kg) or the respective vehicles were given 5 and 10 min before substance administration. Saline (0.9% NaCl) was used. The volume of administration was 5 ml/kg. All doses administered intraperitoneally. Reboxetine mesylate (Tocris Bioscience, Bristol, UK) was administered intravenously, and citalopram HB (Bosche Scientific, New Brunswick, NJ) was administered intraperitoneally. Naloxone HCl (Sigma Chemic, Deisenhofen, Germany) was administered either intravenously or intraperitoneally, and yohimbine HCl and ritanserin HCl (Sigma Chemic) were administered intraperitoneally. Saline (0.9% NaCl) was used as a vehicle except for ritanserin, in which 1% carboxymethyl cellulose in 0.9% NaCl was used. The volume of administration was 5 ml/kg. All doses refer to the respective salt form as indicated above.

Data Analysis

The ED50 calculations were performed according to Litchfield and Wilcoxon (1949). Unless indicated otherwise in the preceding sections, data were analyzed by means of analysis of variance (ANOVA) with or without repeated measures, depending on the experimental design. Significance of treatment effect, time effect, or treatment × time interaction were analyzed by means of Wilks’ lambda statistics. In the case of a significant treatment effect, pairwise comparison was performed at the time of maximal treatment effect by Fisher’s least significant difference test and a post hoc Dunnett test. Results were considered statistically significant if p < 0.05.

Drugs

Tapentadol HCl (Grüenthal GmbH, Aachen, Germany) and morphine HCl (Merck Darmstadt, Germany) were administered either intravenously or intraperitoneally. Reboxetine mesylate (Tocris Bioscience, Bristol, UK) was administered intravenously, and citalopram HBr (Bosche Scientific, New Brunswick, NJ) was administered intraperitoneally. Naloxone HCl (Sigma Chemic, Deisenhofen, Germany) was administered either intravenously or intraperitoneally, and yohimbine HCl and ritanserin HCl (Sigma Chemic) were administered intraperitoneally. Saline (0.9% NaCl) was used as a vehicle except for ritanserin, in which 1% carboxymethyl cellulose in 0.9% NaCl was used. The volume of administration was 5 ml/kg. All doses refer to the respective salt form as indicated above.

Results

Formalin Test, Mouse. Tapentadol showed dose-dependent antinociception [ED50 (95% CI) = 11.3 (6.0–17.9) mg/kg i.p.] in the second phase of the formalin test (F4,45 = 7.02; p < 0.0001). Maximal efficacy of 86.1 ± 7.6% (mean ± S.E.M.) was reached at 31.6 mg/kg i.p. (Fig. 1A; Table 1). Morphine showed dose-dependent efficacy [ED50 (95% CI) = 2.6 (1.8–4.7) mg/kg i.p.] with a maximal effect of 73.7 ± 9.7% at the dose of 4.64 mg/kg i.p. (F3,36 = 7.71; p < 0.0001) (Table 1). The efficacy of ibuprofen was 62.3 ± 10.7 at the dose of 100 mg/kg i.p. with an ED50 of 80.6 (62–100) mg/kg i.p. (F5,36 = 14.25; p < 0.0001) (Table 1).

Formalin Test, Rat. Tapentadol showed a dose-dependent antinociceptive effect [ED50 (95% CI): 9.7 (8.4–11.1)] (F5,36 = 9.96; p < 0.0001). Maximal efficacy of 87.5 ± 7.6% was reached at 14.7 mg/kg i.p. (Fig. 1B). The ED50 (95% CI) of morphine was 1.13 (0.7–1.9) mg/kg i.p. with a maximal effect of 75% at the dose of 4.64 mg/kg i.p. (F5,54 = 232.5; p < 0.0001) (Table 1). Ibuprofen showed 34.2 ± 12.2% antinociceptive efficacy at the dose of 100 mg/kg i.p. (F4,45 = 18.48; p < 0.0001) (Table 1). Higher doses of ibuprofen showed behavioral side effects that might confound the readout, such as decreased motility, and were therefore not further analyzed.

Carrageenan-Induced Inflammatory Pain, Rat. Tapentadol showed dose-dependent inhibition of carrageenan-induced hyperalgesia model and 12 animals in the Carrageenan-induced inflammatory pain model.

Data Analysis

The ED50 calculations were performed according to Litchfield and Wilcoxon (1949). Unless indicated otherwise in the preceding sections, data were analyzed by means of analysis of variance (ANOVA) with or without repeated measures, depending on the experimental design. Significance of treatment effect, time effect, or treatment × time interaction were analyzed by means of Wilks’ lambda statistics. In the case of a significant treatment effect, pairwise comparison was performed at the time of maximal treatment effect by Fisher’s least significant difference test and a post hoc Dunnett test. Results were considered statistically significant if p < 0.05.

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Carrageenan-Induced Inflammatory Pain, Rat. Tapentadol showed dose-dependent inhibition of carrageenan-
induced acute inflammatory pain [ED50 (95% CI): 1.9 (1.7–2.1)] with a maximal effect of approximately 84.2/11006 3.1% at 4.64 mg/kg i.v. (F5,54 = 53.68; p < 0.0001) (Fig. 2). The maximal efficacy of morphine was 81.2/11006 7.1% at the dose of 1.78 mg/kg i.v. The ED50 (95% CI) was 1.2 (1.0–1.3) mg/kg i.v. (F5,54 = 24.68; p < 0.0001) (Table 1). Ibuprofen showed 27.4 ± 4.3% effect at the dose of 100 mg/kg i.p. (F5,54 = 35.93; p < 0.0001) (Table 1). Higher doses showed behavioral side effects (see above).

**CFA-Induced Tactile Hyperalgesia, Rat.** Tapentadol reduced dose-dependently the CFA-induced tactile hyperalgesia with an ED50 (95% CI) of 9.8 (7.8–13.6) mg/kg (Table 1). A maximal efficacy of 70.6/11006 4.8% was reached at 14.7 mg/kg i.p. (F4,45 = 81.1; p < 0.0001) (Fig. 3; Table 1). Morphine showed dose-dependent efficacy [ED 50 (95% CI) 5.9 (5.0–7.1) mg/kg i.p.] with a maximal effect of 69.4/11006 4.8% at the dose of 10 mg/kg i.p. (F4,45 = 4.123; p < 0.006) (Table 1). The efficacy of ibuprofen was 37.6 ± 2.9 at the dose of 100 mg/kg i.p. (F3,36 = 20.41; p < 0.0001). Higher doses showed behavioral side effects (see above).

**CFA-Induced Knee Joint Arthritis, Rat.** Intra-articular CFA injection induced chronic inflammation of the knee joint with a decrease in weight bearing of approximately 50 to 60% after 5 days. This time point of measurement was chosen because of its maximal difference in weight bearing, which lasted for more than 14 days after CFA injection (data not shown). This reduction of weight bearing was dose-dependently reversed by tapentadol with a maximal effect of

<table>
<thead>
<tr>
<th>Model</th>
<th>Treatment</th>
<th>Maximal efficacy (%)</th>
<th>ED50 (95% CI) mg/kg</th>
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<tbody>
<tr>
<td><strong>Formalin Test, Mouse</strong></td>
<td>Tapentadol</td>
<td>84.2 ± 7.6 (31.6; IP)</td>
<td>11.3 (6.0–17.9; IP)</td>
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<td></td>
<td>Morphine</td>
<td>75.2 ± 9.7 (17.9; IP)</td>
<td>2.6 (1.8–4.7; IP)</td>
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<td></td>
<td>Ibuprofen</td>
<td>62.3 ± 10.7 (100; IP)</td>
<td>80.6 (62–100; IP)</td>
</tr>
<tr>
<td><strong>Formalin Test, Rat</strong></td>
<td>Tapentadol</td>
<td>87.5 ± 6.6 (14.2; IP)</td>
<td>11.0 (7.8–18.6; IP)</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td>75.2 ± 11.2 (14.2; IP)</td>
<td>1.13 (0.7–1.9; IP)</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>34.2 ± 1.3 (0.06; IP)</td>
<td>1.0 (ED 25) (0.2–1.7; IP)</td>
</tr>
<tr>
<td><strong>Carrageenan-Induced Acute Pain</strong></td>
<td>Tapentadol</td>
<td>70.6 ± 8.5 (4.64; IV)</td>
<td>9.8 (7.8–13.6; IP)</td>
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<tr>
<td></td>
<td>Morphine</td>
<td>69.4 ± 8.8 (4.64; IV)</td>
<td>1.2 (1.0–1.3; IV)</td>
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<tr>
<td></td>
<td>Ibuprofen</td>
<td>27.4 ± 9.4 (100; IP)</td>
<td>5.9 (5.0–7.1; IP)</td>
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**Table 1** Efficacy and potency of tapentadol and the comparators morphine and ibuprofen in inflammatory pain models. Maximal efficacy (%) at the highest dose that did not show behavioral side effects (e.g. decreased locomotion, sedation, rigor) is shown for the animal models.

IP, intraperitoneal; IV, intravenous; CL, confidence limits; N.C., not counted.

**Fig. 2.** Carrageenan-induced inflammatory pain. Dose-dependent effect of tapentadol (n = 12). *, p < 0.05 versus saline group; ANOVA for repeated measures followed by Fisher’s LSD and post hoc Dunnett tests.

**Fig. 3.** CFA-induced tactile hyperalgesia. Dose-dependent effect of tapentadol (n = 10). *, p < 0.05 versus saline group; ANOVA for repeated measures followed by Fisher’s LSD and post hoc Dunnett tests.
51.0 ± 11.2% at 4.64 mg/kg i.v. (F_{3,28} = 5.05; p < 0.006). The calculated ED_{25} (95% CI) was 0.9 (0.2–1.7) mg/kg i.v. with a maximal effect of 79.0 ± 18.6% at the dose of 100 mg/kg i.p. (F_{2,21} = 7.57; p < 0.003). Higher doses showed behavioral side effects (see above).

**Antagonism Studies.** Antagonism studies were conducted to investigate the relative contribution of opioid and noradrenergic mechanisms of tapentadol in two models of inflammatory pain. Furthermore, the 5-HT_{2} receptor antagonist ritanserin was also used to test whether a serotonergic mechanism was involved in CFA-induced tactile hyperalgesia, an inflammatory pain model in which the SRI citalopram showed a significant effect.

**Carrageenan-Induced Mechanical Hyperalgesia.** Tapentadol showed an effect of 72% at the dose of 2.15 mg/kg i.v., which was reduced to 46% by naloxone (0.3 mg/kg i.p.) (F_{1,22} = 3.322; p < 0.05) (Fig. 5A). Yohimbine (2.15 mg/kg i.p.) reduced the effect of tapentadol from 63% (2.15 mg/kg i.v.) to 14% (Fig. 5B) (F_{1,22} = 64.95; p < 0.001). The combination of the same doses of naloxone (0.3 mg/kg i.p.) and yohimbine (2.15 mg/kg i.p.) abolished the effect of tapentadol (2.15 mg/kg i.v.) (from 77 to −0.6%; F_{1,22} = 283.9; p < 0.001) (Fig. 5C). Naloxone and yohimbine, at the used doses, fully antagonized the effect of the reference compounds morphine (F_{1,22} = 83.27; p < 0.001) and reboxetine (F_{1,22} = 83.56; p < 0.001), respectively (Fig. 5D).

**CFA-Induced Tactile Hyperalgesia.** Tapentadol showed an effect of 68% at the dose of 14.7 mg/kg i.p., which was reduced to 26% by naloxone (0.68 mg/kg i.p.) (F_{1,18} = 36.312; p < 0.001) (Fig. 6A). Yohimbine (2.15 mg/kg i.p.) reduced the effect of tapentadol from 62% at the dose of 14.7 mg/kg i.p. to 41% (F_{3,56} = 128.9; p < 0.001) (Fig. 6B). The 5-HT_{2} receptor antagonist ritanserin (1 mg/kg i.p.) did not reduce the effect of tapentadol (Fig. 6C). The combination of the same doses of naloxone (0.68 mg/kg i.p.) and yohimbine (2.15 mg/kg i.p.)

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**Fig. 4.** Knee joint arthritis model. Dose-dependent normalization of weight bearing by tapentadol (n = 8). *, p < 0.05 versus saline group; ANOVA for repeated measures followed by Fisher’s LSD and post hoc Dunnett tests.

**Fig. 5.** Carrageenan-induced inflammatory pain. Antagonistic effect of naloxone (A) and yohimbine (B), the combination of naloxone and yohimbine on the effect of tapentadol (C), and the effect of naloxone and yohimbine on the effect of morphine and reboxetine (D). A, B, and C, *, p < 0.05 versus saline; #, p < 0.05 versus tapentadol group. D, +, p < 0.001 versus morphine and reboxetine. n = 10 in all groups. ANOVA for repeated measures followed by Fisher’s LSD and post hoc Dunnett tests.
abolished the effect of tapentadol (14.7 mg/kg i.p.) from 70 to 6%; ($F_{1,18} = 53.9; p < 0.001$) (Fig. 6D). The doses of the antagonists naloxone, yohimbine, and ritanserin used in this study fully antagonized the effects of the reference compounds morphine ($F_{1,18} = 79.239; p < 0.001$), reboxetine, and citalopram, respectively (Fig. 6E).

**Discussion**

Tapentadol showed dose-dependent effects in all animal models for inflammatory pain tested in the present study. The compound was effective in acute as well as chronic inflammatory pain. It showed high efficacy with different pain readouts, such as spontaneous pain behavior (formalin-induced flinching, licking, and biting; change in weight bearing), and evoked pain (tactile hyperalgesia, paw pressure). Furthermore, in antagonism studies it was demonstrated that both MOR agonism and NRI contribute to the analgesic effect of tapentadol in inflammatory pain, whereas serotonergic mechanisms do not play a role. This is in accordance with previous studies where tapentadol was found to be effective in a broad range of acute nociceptive and chronic neuropathic pain models, and MOR agonism and NRI were shown to contribute to the efficacy of tapentadol in acute as well as chronic pain (Tzschentke et al., 2007; Schröder et al., 2010). The broad efficacy in various preclinical models for inflammatory pain suggests that tapentadol may also be useful for the treatment of (chronic) inflammatory pain. Indeed, clinical studies have shown that tapentadol has high efficacy and improved tolerability compared with oxycodone in patients with chronic osteoarthritis of the hip or knee (Hale et al., 2009; Lange et al., 2010).

Tapentadol dose-dependently decreased tactile hyperalgesia and showed full efficacy in a carrageenan-induced acute inflammatory response. The application time of tapentadol corresponded with the time point of maximal tactile hyperalgesia (data not shown). However, because tapentadol did not reduce carrageenan-induced paw edema in the same experimental setting as well as with prophylactic treatment (K. Schiene, unpublished data), it is likely that the antihyperalgesic effect of tapentadol was not caused by a genuine anti-inflammatory effect, but rather resulted from its centrally mediated analgesic properties. This interpretation is supported by the finding that, in vitro, tapentadol, at a concentration of 3 μM, showed no interaction with a number of
inflammation targets, such as COX1, COX2, prostanooid and steroid receptors, several cytokines, leukotrienes, and others (H.-H. Hennies and T. Strünker, unpublished data). In the present paw inflammation model, CFA was used to induce a more chronic inflammation compared with the transient acute carrageenan-induced inflammatory response. However, the time point of measurement (1 day after CFA) did not reflect a state of true chronic rheumatoid arthritis, although significant paw swelling and hyperalgesia was already present at this stage. In this model, tapentadol showed full efficacy in reducing tactile hyperalgesia of the inflamed hindpaw, suggesting that the compound has the potential for the treatment of chronic and centralized inflammatory pain.

Both the peripheral and the central nociceptive systems play important roles in the generation of pain after inflammation. At the periphery, nociceptors are sensitized during inflammation. Subsequently, this leads to important central changes, such as central sensitization hypersensitivity. This could also be shown for knee joint inflammation in rats and cats where spinal nociceptive neurons with input from the knee showed an increase of their responses to innocuous and noxious mechanical stimulation of the inflamed joint after an inflammation has developed (Schaible et al.,1987, 2009; Neugebauer and Schaible, 1990). This central sensitization could lead to persistent hypersensitivity of dorsal horn neurons in monoarthritic and polyarthritic rats (Menétrey and Besson, 1982). Tapentadol did not show full efficacy in this model, similar to morphine and ibuprofen. It has to be kept in mind, however, that the behavioral readout in weight bearing is highly sensitive to side effects such as sedation, which can easily prevent a meaningful measurement at higher doses. This could result in putting a low ceiling on the maximal efficacy that can be obtained with this approach, irrespective of the drug being tested. Furthermore, in chronic models, such as the arthritis model, animals may habituate to the guarding behavior, making it partly unresponsive to analgesic drug treatment.

In the present study, tapentadol showed antinociceptive and antihyperalgesic effects in various models of acute and chronic inflammatory pain. This confirms and extends the broad spectrum of analgesic activity shown in previous studies, where tapentadol was effective in a broad range of acute and chronic pain models (Tzschentke et al., 2007, 2009). This analgesic profile is probably the result of a synergistic interaction of the two mechanisms of action of tapentadol, i.e., MOR agonism and NRI (Schröder et al., 2010; Christoph et al., 2011). Previous studies have shown that the relative contribution of MOR agonism and NRI to the analgesic activity of tapentadol depends on the extent of pain chronification. Thus, MOR agonism predominantly mediates tapentadol’s antinociceptive effects in acute pain states, whereas NRI predominantly mediates its antihypersensitive/antiallodynic effects in chronic pain conditions (Schröder et al., 2010).

In the present study, antagonism studies with naloxone and yohimbine were performed only in single-dose experiments. Thus, although the results from these experiments clearly indicate that both mechanisms of action contribute to the analgesic effect of tapentadol in acute and chronic models of inflammatory pain, it is not possible, based on the present data, to estimate the relative contribution of opioid and noradrenergic mechanisms in acute versus chronic inflammatory pain states. Nevertheless, the fact that the in vivo potency difference between tapentadol and morphine was only 1.5–5-fold in the present study (Table 1), despite a 50-fold difference regarding MOR binding affinity in the rat (Tzschentke et al., 2007), strongly suggests a significant contribution of tapentadol’s NRI activity to its overall efficacy in inflammatory pain.

The descending pain modulatory system is an important component in the central sensitization process. The descending pathways originating from the brain stem can be inhibitory or excitatory and are the major mechanisms by which cognitive, attentional, and motivational aspects of the pain experience modulate pain transmission (Millan, 2002). The principal constituents of the descending pain modulatory system are the periaqueductual gray, the rostral ventromedial medulla, and the spinal cord (Millan, 2002). Hyperalgesia in animal models of inflammatory and neuropathic pain is closely linked to the activation of descending modulatory circuits involving both inhibition and facilitation. The activity of these descending circuits is increased during inflammation (Schaible et al., 1991). These major central changes during inflammation are probably the cellular correlates of the behavioral changes and the mechanical and tactile hyperalgesia observed in carrageenan- and CFA-induced inflammatory pain models. In experimental arthritis, the noradrenaline turnover in the spinal cord was reported to be increased, indicating recruitment of descending noradrenergic pain modulatory pathways (Weil-Fugazza et al., 1986). In inflammatory conditions, the antinociceptive potency of systemically and spinally administered α2-adrenoceptor agonists is enhanced (Hylden et al., 1991; Stanfa and Dickinson, 1994), and this can be explained by direct action on the spinal cord (Stanfa and Dickinson, 1994). A study suggested that supraspinal mechanisms may also be involved in the enhanced spinal antihyperalgesic effect of α2-adrenergic drugs in inflamed animals (Molina and Herrero, 2006). Taken together, these findings suggest that in inflammatory pain the noradrenergic descending inhibitory system plays a very important role in endogenous pain modulation, which may well explain why the NRI mechanism of action clearly contributes to the analgesic effect of tapentadol.

In conclusion, tapentadol showed antinociceptive and antihyperalgesic activity in various models of acute and chronic inflammatory pain, and both MOR agonism and NRI were found to contribute to these effects. The data suggest that tapentadol may be a viable treatment option for inflammatory pain conditions.

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Authorship Contributions

Participated in research design: Schiene, De Vry, and Tzschentke. Conducted experiments: Schiene. Performed data analysis: Schiene. Wrote or contributed to the writing of the manuscript: Schiene, De Vry, and Tzschentke.

References


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